# Concurrent Comparison of Two Real-time PCR Assays for the Detection of Zika Virus RNA in Clinical Specimens from an Outbreak

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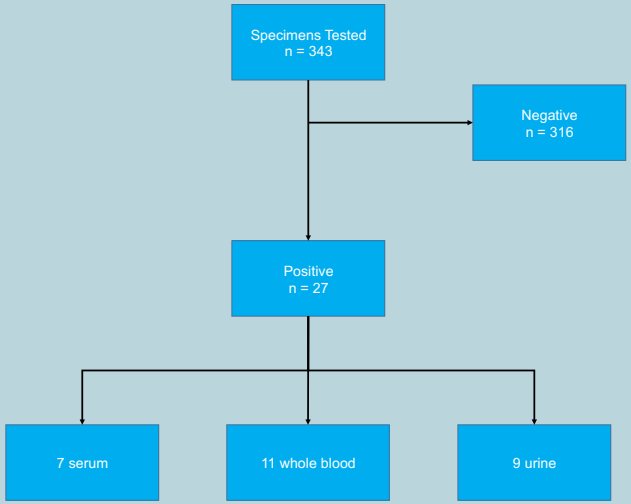
## Abstract

**In the summer of 2016, the State of Florida reported its first cases of autochthonous transmission of Zika virus in the continental United States. In response to the outbreak, the Florida Department of Health's Bureau of Public Health Laboratories (BPHL) provided laboratory support by testing the resulting increased volume of specimens and providing rapid results to public health officials. During the height of the outbreak, two of the three state public health laboratories in Florida (Jacksonville and Tampa) utilized the real-time RT-PCR assay described by Lanciotti and colleagues (2007). The BPHL-Miami implemented the Centers for Disease Control and Prevention's Trioplex Real-time RT-PCR Assay for the simultaneous detection of Zika, Dengue, and Chikungunya viruses. In September of 2016, BPHL-Miami undertook a small study to compare the results of both assays to ascertain whether one performed substantially better than the other. Over a period of two weeks, specimens submitted for testing to the BPHL-Miami were tested using both assays. During this time, 343 whole blood, serum, and urine specimens were received an extracted using two different automated platforms. The resulting extracts were tested on the same day using both RT-PCR assays and the Ct values compared.**




## References

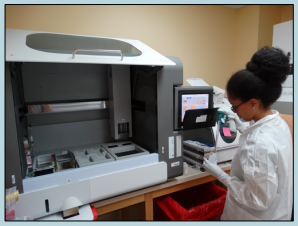
1. Centers for Disease Control and Prevention. (2017). CDC Trioplex Real-time RT-PCR Assay [package insert]. Retrieved from https://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491592.pdf
2. Corman et al. (2016). Clinical Comparison, Standardization and Optimization of Zika Virus Molecular Detection. *Bulletin of the World Health Organization*.
3. Lanciotti et al. (2008). Genetic and Serologic Properties of Zika Virus Associated with and Epidemic, Yap State, Micronesia, 2007. *Emerging Infectious Diseases*.

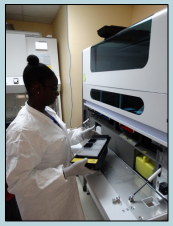
## Methods

**Serum, whole blood, and urine specimens were extracted according to the CDC Trioplex Real-time RT-PCR Assay package insert. To minimize confounding factors, specimens were tested on the same day using the same extracts. Each extract was then tested using the Trioplex assay and the Lanciotti LDT per assay protocols using the ABI 7500 Fast Dx platform. To increase sample size of positive results, archived specimens were reextracted following one freeze-thaw cycle and tested as described.**

**The Trioplex Assay utilizes a single primer/probe set targeting the envelope of the virus. The Lanciotti LDT consists of two primer/probe sets, herein referred to as ZIKV B and ZIKV C. ZIKV B targets a portion of the membrane and envelope proteins, whereas ZIKV C targets the envelope protein. Additionally, the Lanciotti LDT prescribes testing extracts in duplicate, allowing for an equivocal result.**

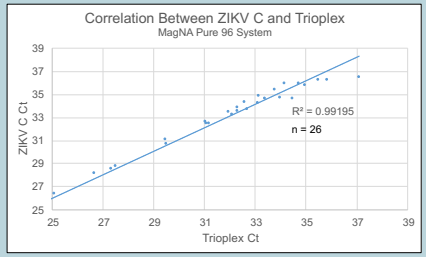
| Extraction Method | Throughput | Extraction Kit | Initial Specimen Volume | Elution Volume | Comments |
|---|---|---|---|---|---|
| MagNA Pure LC 2.0 | 32 specimens/ ~2 hours | Total Nucleic Acid Isolation Kit | 200 µL | 100 µL for blood 60 µL for others | Ideal for small volumes of specimens |
| MagNA Pure 96 | 96 specimens/ ~hour | DNA and Viral NA Small Volume Kit | 200 µL | 100 µL | Ideal for surge |

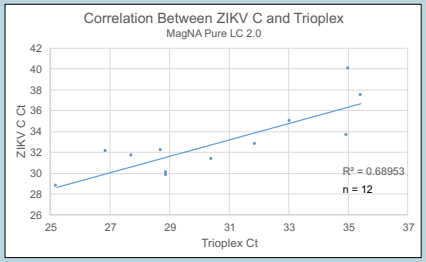




| | | CDC Trioplex Assay | | Lanciotti LDT | |
|---|---|---|---|---|---|
| | | Quanta qScript One-Step qRT-PCR Kit, Low Rox | | QIAGEN QuantiTect Probe RT-PCR Kit | |
| Cycling Parameters | cDNA Synthesis | 50°C | 30 min | 50°C | 30 min |
| | Activation | 95°C | 5 min | 95°C | 15 min |
| | 45 Cycles | 95°C | 15 sec | 95°C | 15 sec |
| | | 60°C | 1 min | 60°C | 1 min |
| Concentration | Forward Primer | 50 µM | | 100 µM | |
| | Reverse Primer | 50 µM | | 100 µM | |
| | Probe | 7.5 µM | | 25 µM | |
| Master Mix Recipe | Water | 0.5 µL | | 6.6 µL | |
| | 2X Mix | 12.5 µL | | 12.5 µL | |
| | Forward Primer | 0.167 | | 0.25 µL | |
| | Reverse Primer | 0.167 | | 0.25 µL | |
| | Probe | 0.167 | | 0.15 µL | |
| | Enzyme Mix | 0.5 µL | | 0.25 µL | |
| | Template | 10 µL | | 5 µL | |

## Results




Discordant Results

| MagNA Pure 96 | | Trioplex Assay | | Lanciotti LDT Assay | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ID | Specimen Type | Trioplex Ct | Interpretation | ZIKV B Ct | Interpretation | ZIKV C Ct | Interpretation | Final Determination | Epidemiological Determination |
| MSV16002932 | Serum | 37.19 | Detected | Undet. | Not Detected | NP | N/A | Not Detected | Not a Case |
| MSV16002934 | Serum | 37.49 | Detected | 39.24* | Not Detected | NP | N/A | Not Detected | Case |
| MSV16003020 | Serum | 37.01 | Detected | Undet. | Not Detected | NP | N/A | Not Detected | Case |
| MSV16003028 | Whole Blood | Undet. | Not Detected | 37.22 | Detected | Undet. | Not Detected | Equivocal | Case |
| MSV16003100 | Serum | Undet. | Not Detected | 38.19 | Equivocal | Undet. | Not Detected | Equivocal | Case |
| MSV16003156 | Whole Blood | 37.69 | Detected | Undet. | Not Detected | NP | N/A | Not Detected | Case |
| MSV16003189 | Whole Blood | Undet. | Not Detected | 37.95* | Equivocal | Undet. | Not Detected | Equivocal | Not a Case |
| MSV16003221 | Serum | 36.28 | Detected | 38.57* | Not Detected | NP | N/A | Not Detected | Case |

| MagNA Pure LC 2.0 | | Trioplex Assay | | Lanciotti LDT Assay | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ID | Specimen Type | Trioplex Ct | Interpretation | ZIKV B Ct | Interpretation | ZIKV C Ct | Interpretation | Final Determination | Epidemiological Determination |
| MSV16002381 | Serum | 32.59 | Detected | Undet. | Not Detected | NP | N/A | Not Detected | Case |
| MSV16002939 | Whole Blood | Undet. | Not Detected | 37.97* | Equivocal | NP | N/A | Equivocal | Case |

Reflected ZIKV B and ZIKV C values are averages between replicates. An asterix denotes one replicate was undetermined (no amplification).

## Discussion

**The correlation between the ZIKV C and Trioplex assays are fairly similar. However, this correlation appears to be dependent on the extraction method- the specimens extracted with the MagNA Pure 96 System exhibit a much higher correlation than those extracted by the MagNA Pure LC 2.0 instrument. This trend was also seen with the ZIKV B primer/probe set (data not shown), although this is also likely influenced by the different targets.**

**As might be expected, the Ct values diverge as the Ct values increase. This is likely due to differences in each assay's limit of detection.**

**A paired t-test was used to determine if the Ct values between the ZIKV C and Trioplex assays were significantly different when extracted using the MagNA Pure 96. Trioplex Ct values were consistently lower (26 out of 27) with a mean difference between ZIKV C of 1.16 ($\sigma = 0.54$), $p < 0.001$.**

**Several limitations to this study include a limited sample size of ZIKV-positive specimens. These assays were also not performed quantitatively, so interpretation of Ct values must be done so with caution.**